### REVIEW



Regarding: Doctoral dissertation submitted to obtain the PhD degree in Plant Physiology

Name of PhD student: Dobrinka Anastasova Balabanova-Ivanovska, Assist.Professor in the Department of Plant Physiology and Biochemistry in Agricultural University of Plovdiv

Title of PhD thesis: Physiological responses of sunflower Clearfield hybrids to the herbicide imazamox

**Prepared the review:** Prof. Dr Andon Vassilev (one of the supervisors of the PhD student), Department of Plant Physiology and Biochemistry, member of the scientific jury according the Order № RD-16-1010 of 24. October 2016 of the Rector of Agricultural University of Ploydiv

1. Actuality of the addressed problem

Sunflower is one of the most important oil crops in the world. Sunflower yield is strongly dependent on weeds competition, especially in the early stages of plant development. The Clearfield technology, based on both high selective herbicide imazamox and imazamox-tolerant hybrids (IMI-R), is very efficient in weed control, but still needs improvement. Although the high selectivity of IMI-R sunflower hybrids, transition crop injuries occasionally appear due to higher imazamox rates, application timing as well as unsuitable environmental conditions. This problem attracts scientific attention, namely on incomplete information about the mechanisms of herbicide degradation in sunflower plants as well as some possibilities to ameliorate temporary imazamox-induced crop injuries. Therefore, I think the chosen research topic of the dissertation is very actual from both theoretical and applied viewpoint.

2. Aim, tasks, hypothesis and scientific methods

The aims of the conducted study is (I) to reveal the degradation rate and physiological effects of the herbicide imazamox in IMI-resistant and IMI-susceptible sunflower hybrids and (II) to examine the possibility of reducing the temporary growth inhibition caused on herbicide imazamox on sunflower plants by foliar application of amino acid extract.

To achieve the objectives five logically connected tasks have been set. The experimental design of each task has been properly chosen including hydroponics and pot soil experiments, different imazamox rates as well as different way of additives application such as root application of branch chain amino acids (BCAA) and leaf application of biostimulant (AAE). The used scientific methods - physiological, biophysical, biochemical, chemical and molecular are suitably chosen giving an opportunity the studied problem to be highlighted at different levels of biological organization – from molecule to plant. The motivation to conduct task 5 is based on a logical working hypothesis, namely that could be possible to ameliorate plant performance of imazamox-treated sunflower plants by the combined application of imazamox and biostimulant having amino acid content origin. My opinion is that the conducted studies are correctly performed from the methodological viewpoint.

## 3. Content of the thesis

The thesis starts with literature review based on 199 references. It is firmly well written, well-structured and provides up to date information on the studied problem. The chapter materials and methods give comprehensive information about the objects (sunflower hybrids; chemical products) and the used scientific methods, including statistical treatment of the results.

The results obtained are presented in 5 chapters. The studies start with an evaluation of the tolerance of five IMI-R sunflower hybrids to imazamox treatment. Using as criteria selected parameters of plant photosynthetic performance at both herbicide-induced stress period and recovery period, the hybrid Mildimi was evaluated as the most tolerant and further used in the following experiments. To understand to what extent non-specific and specific responses are involved in sunflower plant response to imazamox, fact responses of IMI-R and IMI-S hybrids were compared after 24 hours. Differences were found, namely: antioxidative defence enhancement in IMI-S plants and herbicide detoxification in IMI-R plants. In the next step of the study, the plant performance modulation of IMI-R hybrid Mildimi has been monitored by complex of physiological, biochemical, molecular and morphological analyses. It was found the imazamox, applied in recommended rate, was able to induce a transition inhibition of photosynthetic functioning as well as plant growth retardation, which were almost fully recovered 14DAT. To understand to what extent the disturbed protein turnover is the main reason for the observed herbicide-induced physiological disorders, the influence of BCAA supplementation of the growth medium on plant physiological performance has been studied. The observed improvement of both photosynthetic performance and growth of imazamox-treated plants proved the mode of action of this herbicide. In the final step, the effect of leaf applied biostimulant (amino acid extract) together with imazamox application on IMI-R plants have been evaluated. The results demonstrated positive influence of the combined application, which motivates next field investigations to develop this possibility to practical implementation.

# 4. Results, conclusions and achievements

The results obtained are presented in 15 composite figures and 4 tables. In addition, the included 3 photographs improve our evidence for the influence of both single and combined application of imazamox and additives (BCAA and biostimulant). The results are commented competently with rational use of the presented information in the literature survey.

The main results are summarized in 9 conclusions, which I fully accept because they correctly reflex the results obtained. Due to the limited volume of the review I will comment only on the achievements, according to my viewpoint.

- I accept the obtained evidence that the metabolite glutathione participates in the herbicide imazamox detoxification in sunflower plants by conjugation reactions catalyzed by the glutathione S-transferase enzymes family is a novel fact. This fact complements the known detoxification pathway of imazamox in plants by cytochrome P<sub>450</sub> monooxygenase and glycosyl transferases.
- I think that the established facts concerning the photosynthetic performance of imazamox-treated sunflower plants, namely imazamox-induced decrease of

both concentration of active PSII reaction centers and the relative part of active reaction centers compared to the total chlorophyll content are novel, original facts, which detailed the overall view of imazamox-photosynthesis interactions.

• I accept that the conducted monitoring study on the physiological responses of sunflower IMI-R plants subjected to both separate and combined application of imazamox and branched chain amino acids enrich the overall picture of imazamox-induced injuries, their consequences and recovery of the plants, therefore could be characterized as a study with an innovative character.

# 5. Critical notes, comments and questions

I have no critical comments. As one of the supervisors of the PhD candidate Dobrinka Balabanova, I had an opportunity to discuss with her broad aspects of questions, starting with the plan of experiments, discussion of the results as well as structure and preparation of the thesis. The PhD student received significant support from all supervisors in each step of the studies, but the experimental work, writing of thesis and preparation of the publications are her own.

### 6. Publications

A part of the results in this study was published in two scientific journals – Agricultural Science (Bulgarian journal) and an international peer reviewed journal Frontiers in Plant Science. The publication in Frontiers in Plant Science is a good indicator for the high quality of the performed research.

### 7. CONCLUSION

The PhD candidate Dobrinka Balabanova learned a broad number of methods in Plant Sciences and implemented them in the performed studies in this PhD thesis. She obtained significant theoretical knowledge helping her to discuss and integrate the results obtained at different plant biological levels. Furthermore, Dobrinka Balabanova demonstrated capacity to communicate her results with scientific community at national and international conferences. According to my viewpoint, she is now able to conduct independent research in the field of Plant Physilogy and Biochemistry.

My conclusion is that the presented PhD thesis has high scientific quality and meets the requirements of both the Law on Development of the Academic Staff in the Republic of Bulgaria and its Regulations of the Agricultural University of Plovdiv.

According to the requirements, I evaluated POSITIVELY the thesis and would like to propose to the honorary scientific jury to vote also positively and to confer a PhD degree in Biology (Plant Physiology) to Dobrinka Anastasova Balabanova-Ivanovska.

Date: 17.11.2016

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(Prof. Dr. Andon Vassilev)